As one example of the use of the term "synthetic oligonucleotide" in the art, The McGraw-Hill Encyclopedia of Science & Technology (McGraw-Hill, 1997, volume 12, page 358) states in an article about oligonucleotides:

> ... deoxyribooligonucleotides and ribooligonucleotides of defined sequence can be obtained by using restriction endonucleases and ribonucleases, respectively. ...Chemical synthesis of oligonucleotides is, however, the preferred procedure for preparing a deoxyribooligonucleotide or ribooligonucleotide of defined sequence. The sequence is usually completed on silica gel or glass, where the first nucleotide is joined covalently to these inorganic matrices. Additional nucleotides are chemically added sequentially to the first in order to form the oligonucleotide of defined sequence. The synthetic oligonucleotide is removed from the support, purified, and used for various biochemical experiments. (emphasis added)

This reference clearly distinguishes between oligonucleotides formed enzymatically and those formed by chemical synthesis, and refers to the latter as "synthetic oligonucleotides."

As another example of the use of the term "synthetic oligonucleotide" in the art, the textbook Molecular Biology of the Cell, Third Edition (Garland Publishing, 1994, page 305) states:

> [a]t the same time that microbiologists were developing DNA cloning techniques, organic chemists were improving the methods for synthesizing short DNA chains. Today, such synthetic DNA oligonucleotides are routinely produced by machines that can automatically synthesize any DNA sequence up to 120 nucleotides long overnight. (emphasis added)

Hence, the latter reference also identifies "synthetic oligonucleotides" as oligonucleotides prepared by chemical synthesis. Copies of the relevant portions of these two references are included with this response as Attachment A and Attachment B.

In contrast, in a section of the Office Action entitled "Response to Arguments" (page 4) the Examiner constructed a definition of "synthetic oligonucleotide" from a definition of "synthetic" found in the Academic Press Dictionary of Science and Technology. The definition cited by the Examiner defines synthetic to mean "any product or item that is the result of human technology rather than something that exists in nature." Based on this definition, the Examiner concluded that

> [t]he labeled cDNA and the labeled genomic DNA of Lashkari are products that result from human technology and do not exist in nature.... Therefore, the labeled cDNA and the labeled genomic DNA are encompassed by the claimed "synthetic oligonucleotides."

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Applicant respectfully submits that the Examiner has misconstrued the term "synthetic oligonucleotide." As demonstrated above, "synthetic oligonucleotide" has a clear meaning to one of ordinary skill in the art. Hence, it is unnecessary and inappropriate to construct a definition for "synthetic oligonucleotide" by juxtaposing a definition of "synthetic" with an (implicit) definition of "oligonucleotide" as the Examiner has done.

Moreover, even the dictionary cited by the Examiner supports Applicant's construction of "synthetic oligonucleotide." The dictionary cited by the Examiner provides three definitions of "synthetic." The Examiner selected the definition indicated by the dictionary to be appropriate for engineering. The same dictionary provides an alternate definition of "synthetic" that it indicates is appropriate for chemistry: "relating to compounds formed artificially by chemical synthesis." Since oligonucleotides are chemical compounds, the latter definition is the appropriate definition. Using the latter definition, "synthetic oligonucleotides" as recited in Claim 1 are oligonucleotides "formed artificially by chemical synthesis."

The Examiner asserts in paragraph 3 of the Office Action that Lashkari et al. discloses "hybridizing the microarray with a mixture of labeled synthetic oligonucleotides" at page 13058, left column, second paragraph. The cited and adjacent portions of Lashkari et al. disclose hybridizing an array with cDNA or with genomic DNA. One of ordinary skill in the art would recognize that cDNA and genomic DNA are prepared by enzymatic methods, rather than by chemical synthesis. For example, the paragraph of Lashkari et al. cited by the Examiner describes preparation of genomic DNA using Klenow enzyme. Hence, as demonstrated above, one of ordinary skill in the art would not regard cDNA and genomic DNA as "synthetic oligonucleotides."

The "synthetic oligonucleotides" recited in Claim 1 are also distinguishable from cDNA and genomic DNA by molecular size. One of ordinary skill in the art would recognize that the term "oligonucleotide" typically refers to a polymer containing less than about 100 nucleotides. For example, the encyclopedia article included as Attachment A and cited above states on page 357 that "[f]ragments containing up to 50 nucleotides are generally termed oligonucleotides...." In contrast, one of ordinary skill in the art would recognize that cDNA and genomic DNA materials typically contain 100 to 5000 nucleotides. Hence, such cDNA and genomic DNA materials are not properly described as oligonucleotides, much less as "synthetic oligonucleotides."

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For the above reasons, Claim 1 is patentable over Lashkari et al. Claims 8, 9, 12, 14, 17, 18, 21 and 24, directly or indirectly dependent on Claim 1, are patentable over Lashkari et al. for at least the reasons for which Claim 1 is patentable over Lashkari et al.

Claims 1, 5, 8, 11, 12, 14, 17, 18, 21, 24, and 26 are rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Brown et al. (U.S. Patent No. 5,807,522). Applicant respectfully traverses this rejection.

The Examiner states in paragraph 5 of the Office Action that Brown et al. discloses

> ...hybridizing the microarray with a mixture of labeled synthetic oligonucleotide i.e. cloned DNA fragments wherein the mixture comprises oligonucleotides complementary to the genomic segments....

The passage in Brown cited by the Examiner to support his statement reads in part:

> [a] mixture of the labeled cDNAs from the two cell types is added to an array of polynucleotides representing a plurality of known genes derived from the two cell types, under conditions that result in hybridization of the cDNAs to complementary-sequence polynucleotides in the array. (column 4, line 60 to column 5, line 8, emphasis added)

As demonstrated above, cDNAs as disclosed by Brown et al. are distinguishable from the "synthetic oligonucleotides" recited in Claim 1. Hence the disclosure of Brown et al. does not anticipate Claim 1.

The obviousness rejection of Claim 1 over Brown et al. was directed to the portion of Claim 1 which recites "amplifying a plurality of genomic segments" rather than to the portion which recites "hybridizing the microarray with a mixture of labeled synthetic oligonucleotides...." Since Brown et al. does not teach or suggest "hybridizing the microarray with a mixture of labeled synthetic oligonucleotides," the Examiner has not met the requirements of a *prima facie* case of obviousness as set forth, for example, in the MPEP §2142.

For the above reasons, Claim 1 is patentable over Brown et al. Claims 5, 8, 11, 12, 14, 17, 18, 21, and 24, directly or indirectly dependent on Claim 1, are patentable over Brown et al. for at least the reasons for which Claim 1 is patentable over Brown et al.

Independent Claim 26 also recites "hybridizing the microarray with a mixture of labeled synthetic oligonucleotides...." Hence Claim 26 is patentable over Brown et al. for at least the reasons for which Claim 1 is patentable over Brown et al.

Claims 3, 4, 6, 7, 9, and 10 are rejected under 35 U.S.C. §103(a) as obvious over Brown et al. This rejection is respectfully traversed. Claims 3, 4, 6, 7, 9, and 10, directly or

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indirectly dependent on Claim 1, are patentable over Brown et al. for at least the reasons for which Claim 1 is patentable over Brown et al.

Claims 13, 15, 16, and 25 are rejected under 35 U.S.C. §103(a) as obvious over Brown et al. in view of Wang et al. This rejection is also respectfully traversed. Wang et al. does not remedy the defects of Brown et al. with respect to Claim 1. Consequently, Claim 1 is patentable over Brown et al. in view of Wang et al. Claims 13, 15, 16, and 25, directly or indirectly dependent on Claim 1, are patentable over Brown et al. in view of Wang et al. for at least the reasons for which Claim 1 is patentable over this combination.

Claims 19-20 and 22-23 are rejected under 35 U.S.C. §103(a) as obvious over Brown et al in view of Fodor et al. This rejection is respectfully traversed. Fodor et al. does not remedy the defects of Brown et al. with respect to Claim 1. Hence, Claim 1 is patentable over Brown et al. in view of Fodor et al. Claims 19-20 and 22-23, directly or indirectly dependent on Claim 1, are patentable over Brown et al. in view of Fodor et al. for at least the reasons for which Claim 1 is patentable over this combination.

For the above reasons, Applicant respectfully requests reconsideration and allowance of Claims 1 and 3-26. Should the Examiner have any questions concerning this response, the Examiner is invited to call the undersigned at (408) 453-9200.

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Attachment A

McGRAW-HILL ENCYCLOPEDIA OF Science & Technology

12

NIO-OZO

8th Edition

An international reference work in twenty volumes including an index

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Classification. The Oligochaeta have been considered with the Polychaeta as an order of the Archetopoda, and with the Hirudinea as an order of the Clitellata. The nature of the reproductive system and the absence of parapodia sharply set the oligochaetes apart from the polychaetes. The Hirudinea are much closer to the oligochaetes but constitute a homogeneous group long considered a separate class. The Oligochaeta therefore are usually treated as a class of the phylum Annelida, coordinate in rank with the Polychaeta and Hirudinea as previously proposed by G. Pickford. Following Michaelsen, there are four orders of the class: (1) Plesiopora plesiotheca, micronephridiostomal, male pores on the segment following the testes, and spermathecae in the region of the genital segments; (2) Plesiopora prosotheca, as for the first order, except the spermatheca are a number of segments in front of the genital segments; (3) Prosopora, mesonephridiostomal, male pores in the segment of the posterior testes; and (4) Opisthopora, meganephridiostomal, male pores opening posteriorly to last testicular segment. Holt has removed the Branchiobdellidae from the Oligochaeta and proposed that the Oligochaeta, Branchiobdellida, Acanthobdellida, and Hirudinea be considered orders of the class Clitellata.

That the oligochaetes are descended from some fine polychaetelike ancestors seems certain, but there is no agreement as to the relationships of families within the class. Michaelsen regarded the Aeolosatidae as primitive; Stephenson, the Lumbriculidae; no definite solution to this question has been reached. *See* ANNELIDA. Perry C. Holt

Bibliography. P. P. Grasse, *Traité de Zoologie*, vol. 5, 1959; M. S. Laverack, *The Physiology of Earthworms*, 1963; W. Michaelsen, Oligochaeta, in H. G. Kükenthal and T. Krumbach, *Handbuch der Zoologie*, vol. 2, 1928–1930; S. P. Parker (ed.), *Synopsis and Classification of Living Organisms*, 2 vols., 1982; J. Stephenson, *The Oligochaeta*, 1930.

Oligoclase

A plagioclase feldspar with composition in the range $Ab_{90}An_{10}$ to $Ab_{70}An_{30}$, where Ab represents the composition of albite, $NaAlSi_3O_8$, and An represents the composition of anorthite, $CaAl_2Si_2O_8$. The diagnostic properties are hardness on Mohs scale, 6–6.5; density, 2.65 g/cm³; two good cleavages that intersect at approximately 90°; and color usually white or colorless, transparent to translucent. The presence of minute, mutually parallel inclusions of hematite (Fe_2O_3) causes a golden play of color in the variety of oligoclase called aventurine or sunstone. Repeated twinning is common and results in closely spaced striations visible with a hand-held magnifier. Mean refractive index is 1.545; the mineral may be optically positive or negative, depending on composition. Oligoclase is triclinic. The mineral is common in both plutonic and volcanic silicic igneous rocks, as well as in quartzofeldspathic and pelitic metamorphic rocks. *See* ALBITE; ANORTHITE; CRYSTAL STRUCTURE.

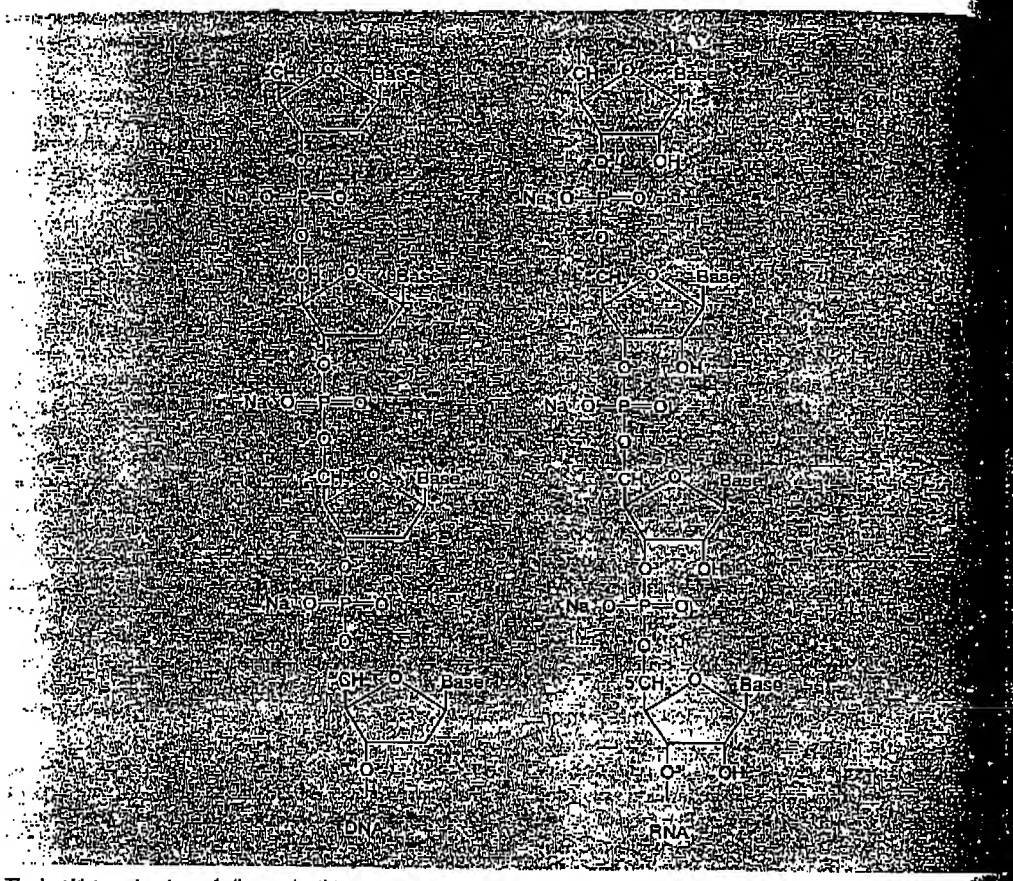
Like all feldspars, oligoclase is a tectosilicate. Each aluminum (Al) and each silicon (Si) atom is surrounded by four oxygen atoms that form a tetrahedron around it; each tetrahedron shares the four oxygen atoms at its corners with four other tetrahedra, forming a three-dimensional atomic framework containing open voids that accommodate the sodium-calcium (Na,Ca) atoms. At the highest temperatures typical of igneous processes, the Al and Si atoms are statistically disordered among the symmetrically distinct tetrahedra. As cooling proceeds, Al and Si order preferentially into symmetrically different tetrahedra. If the composition is more Ab-rich than about $Ab_{83}An_{17}$, then exsolution or spinodal decomposition to an intergrowth of $Ab_{76\pm6}An_{24\pm6}$ and completely ordered $Ab_{99\pm1}An_{1\pm1}$ also occurs. The more calcic lamellae of this so-called peristerite intergrowth are e-plagioclase, consisting of alternating nanometer-scale lamellar domains of albitelike and anorthitelike structure. Oligoclases with bulk compositions more calcic than $Ab_{83}An_{17}$ do not form peristerite intergrowths, but they nonetheless develop into e-plagioclases upon cooling. *See* FELDSPAR; IGNEOUS ROCKS; METAMORPHIC ROCKS. Dana T. Griffen

Bibliography. W. A. Deer, R. A. Howie, and J. Zussman, *An Introduction to the Rock-Forming Minerals*, 2d ed., 1992; D. T. Griffen, *Silicate Crystal Chemistry*, 1992; J. V. Smith and W. L. Brown, *Feldspar Minerals*, 2d ed., 1988.

Oligonucleotide

A deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequence composed of two or more covalently linked nucleotides. Oligonucleotides are classified as deoxyribooligonucleotides or ribooligonucleotides. Fragments containing up to 50 nucleotides are generally termed oligonucleotides, and longer fragments are called polynucleotides. *See* DEOXYRIBONUCLEIC ACID (DNA); RIBONUCLEIC ACID (RNA).

Composition. A nucleotide is composed of a purine or pyrimidine base, a 5-carbon sugar, and a phosphate group. A deoxyribooligonucleotide consists of a 5-carbon sugar called deoxyribose joined covalently to phosphate at the 5′ and 3′ carbons of this sugar to form an alternating, unbranched polymer (see **illus.**). A purine or pyrimidine base is linked through one of its nitrogens by an *N*-glycosidic bond to the 1′ carbon of deoxyribose. The four bases found in a deoxyribooligonucleotide are guanine, cytosine, adenine, and thymine. A ribooligonucleotide consists of a similar repeating structure where the 5-carbon sugar is ribose. The four bases found in a ribooligonucleotide are guanine, cytosine, adenine, and uracil. The sequence or ordering of these bases determines the



The backbone structure of oligonucleotides as the sodium salt; actual oligonucleotides are 20–50 bases in length. Bases may be thymine, uracil, cytosine, guanine, or adenine.

biological function of the deoxyribooligonucleotide or ribooligonucleotide.

Preparation. Oligonucleotides can be derived from high-molecular-weight DNA and RNA by acid, base, or enzymic hydrolysis. Enzymic hydrolysis is most useful, since deoxyribooligonucleotides and ribooligonucleotides of defined sequence can be obtained by using restriction endonucleases and ribonucleases, respectively. These enzymes recognize certain DNA or RNA sequences and hydrolyze the phosphate-sugar ester bonds, thereby freeing particular oligonucleotides from the polynucleotides. Chemical synthesis of oligonucleotides is, however, the preferred procedure for preparing a deoxyribooligonucleotide or ribooligonucleotide of defined sequence. The sequence is usually completed on silica gel or glass, where the first nucleotide is joined covalently to these inorganic matrices. Additional nucleotides are chemically added sequentially to the first in order to form the oligonucleotide of defined sequence. The synthetic oligonucleotide is removed from the support, purified, and used for various biochemical experiments.

See RESTRICTION ENZYME.

Uses. Oligonucleotides having biological activity in living organisms are primarily constituents of high-molecular-weight DNA or RNA. They include sequences 2–50 nucleotides in length that are recognized by repressors, activator proteins, polymerases, repair enzymes, and the translation machinery of the cell. There are, however, certain oligonucleotides which are not constituents of high-molecular-weight DNA or RNA but which are also important to the cell. For example, small oligonucleotides of RNA (10–20 nucleotides in length) are essential for priming the replication of DNA. This replication process also generates DNA segments called Okazaki fragments which contain 50–1000 nucleotides and are sometimes considered deoxyoligonucleotides. These Okazaki fragments are then joined to form high-molecular-weight DNA. A ribooligonucleotide called (2′–5′)$_n$A [where n extends from 1 to about 15] is produced in cells in response to interferon induction and appears to be a mediator of interferon antiviral action. This ribooligonucleotide contains 2′ and

sphate diester bonds rather than the normal 3' 5' linkages, and has only adenine as the base.

GENE ACTION.

hemically synthesized oligonucleotides of pre-ermined sequence have proven to be very use-for studying a large number of biochemical cesses. In the 1960s, these compounds were d to decipher the genetic code. Later, chemi-ly prepared deoxyoligonucleotides were joined form genes for transfer RNAs. Gene synthesis m synthetic deoxyoligonucleotides is now rou-ely used to prepare genes and modified genes proteins having potential clinical applications. igonucleotides have also been used to diagnose netic disorders and bacterial or viral infections.

GENE; GENETIC CODE; GENETIC ENGINEERING; CLEIC ACID.

Marvin H. Carruthers

Bibliography. Z. Estrov, R. Kurzrock, and M. lpaz, *Interferons: Basic Principles and Clinical pplications*, 1993; K. Itakura et al., Synthesis d use of synthetic oligonucleotides, *Annu. Rev. ochem.*, 53:323–356, 1984; H. G. Khorana, Total nthesis of a gene, *Science*, 203:614–625, 1979; G. Khorana et al., Polynucleotide synthesis and genetic code, *Cold Spring Harbor Symp. Quant. ol.*, 31:39–49, 1966; J. D. Watson, *Molecular ology of the Gene*, 4th ed., 1987.

ligopygoida

order of irregular echinoids in the superorder cognathostomata resembling clypeasteroids but ding the accessory ambulacral pores charac-istic of that group. Oligopygoids have well-veloped petals, and there are characteristic small miplates present below the petals. These demi-tes are wedge-shaped and usually do not reach inner surface of the test. Each has a simple mbulacral pore. The apical disk is monobasal d the mouth oval and usually deeply sunken. igopygoids have a lantern, which closely resem-s that of clypeasteroids, and their lantern muscle-achment structures are a mixture of ambulacral d interambulacral processes.

here are two genera, *Oligopygus* and *Haimea*, maining about 25 species, all from the middle d upper Eocene of the Caribbean and Gulf Mexico regions. They were probably infaunal posit feeders like present-day laganiids. *See* HINODERMATA; NEOGNATHOSTOMATA.

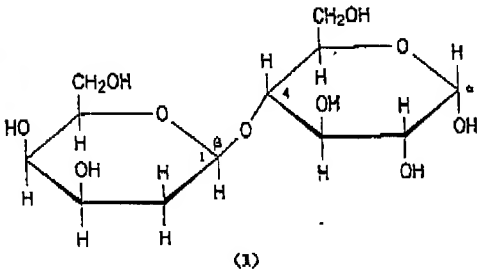
Andrew B. Smith

Bibliography. A. B. Smith, *Echinoid Palaeobiology*, 984.

ligosaccharide

carbohydrate molecule composed of 3–20 nosaccharides. Generally, free oligosaccharides not quantitatively constitute a significant proportion of naturally occurring carbohydrates. Most carbohydrates that occur in nature are in the form of monosaccharides (such as blood sugar, or glucose), disaccharides (such as table sugar, or sucrose, and milk sugar, or lactose), and polysaccharides (such as starch and glycogen, polyglucose molecules, or chitin). *See* GLUCOSE; LACTOSE; MONOSACCHARIDE; POLYSACCHARIDE.

Composition. The monosaccharides of multiple sugar units such as disaccharides, oligosaccharides, and polysaccharides are connected with each other through bonds called glycosidic linkages. Monosaccharides are generally classified as aldose (an aldehyde sugar) or ketose (a ketone sugar). Chemically, these sugar molecules are found mainly as cyclic molecules, which are referred to as hemiacetals. Cyclic hemiacetals exist as five-membered rings (called furanoses) or six-membered rings (called pyranoses). Both classes of sugars are reducing sugars, meaning that the aldehyde or ketone group can become oxidized and in turn will reduce certain compounds. Simple sugars (monosaccharides) are linked primarily to other sugars and to other molecules through these reducing groups. For example, lactose (1) has the structure of galactosyl-



(1)

1,4-glucose, meaning that carbon-1 of galactose is linked to carbon-4 of glucose. The proper name for this structure of lactose is β-D-galactopyranosyl-(1-4)-α-D-glucopyranose. In ring structures, the aldehyde or ketone carbon is referred to as the anomeric carbon, and the cyclized aldose or ketose exists in one of two anomeric configurations designated as α or β. The complexity of oligosaccharide chemistry is appreciated when the possible combinations are considered in forming a disaccharide between galactose and glucose. *See* GALACTOSE.

Carbon-1 of galactose can be linked to any of the hydroxyl groups of glucose, giving four possible disaccharides. Since carbon-1 of galactose exists as the anomeric carbon (α or β), the number is multiplied by 2, so that there are eight possible structures based only on these assumptions. The number of possible isomeric oligosaccharides that could exist for a trisaccharide composed of three different monosaccharides with the typical structure given in (1) is 1056. If the reducing group of one monosaccharide is linked to the reducing group of

Attachment B

MOLECULAR BIOLOGY OF THE CELL

THIRD EDITION

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

coinherited by each progeny (*linked*). By screening large family groups for the coinheritance of a gene of interest (such as one associated with a disease) and a large number of individual RFLPs, a few RFLP markers can be identified that are unambiguously coinherited with the gene (this requires the analysis of many individuals, as explained in Figure 7–16). DNA sequences that surround the gene can thereby be located. Eventually the DNA corresponding to the gene itself can be found by techniques to be described later (see Figure 7–30). Many genes that cause human diseases are being isolated in this way, allowing the proteins they encode to be analyzed in detail.

It is clear from Figure 7–16 that the closer an RFLP marker is to the mutant gene of interest, the easier it is to locate the gene unambiguously. To facilitate these and other studies, an intensive effort is underway to prepare a *high-resolution RFLP map* of the human genome, with thousands of RFLP markers spaced an average of 10^5 nucleotide pairs apart. On average, two markers separated by this distance will be coinherited by 99 of every 100 progeny. Such a map will make it relatively easy to use genetic linkage studies to locate a gene that has been identified only by the effect of a mutation in humans, allowing it to be mapped to one or a few large DNA clones in an ordered genomic DNA library. Isolation of the gene might then be accomplished relatively quickly.

Synthetic DNA Molecules Facilitate the Prenatal Diagnosis of Genetic Diseases [12]

At the same time that microbiologists were developing DNA cloning techniques, organic chemists were improving the methods for synthesizing short DNA chains. Today, such synthetic *DNA oligonucleotides* are routinely produced by machines that can automatically synthesize any DNA sequence up to 120 nucleotides long overnight. This ability to produce DNA molecules of a desired sequence makes it possible to redesign genes at will, an important aspect of genetic engineering, as explained later. Such synthetic oligonucleotides can also be used as labeled probes to detect corresponding genomic sequences by DNA hybridization. By varying the temperature at which the hybridization reaction is run, it is possible to vary the *stringency* of the hybridization: above a certain temperature, only perfectly matched sequences will hybridize, and this can make it possible, for example, to detect a mutant gene in the prenatal diagnosis of genetic disease.

More than 3000 human genetic diseases are attributable to single-gene defects. In most of these the mutation is recessive: that is, it shows its effect only when an individual inherits two defective copies of the gene, one from each parent. One goal of modern medicine is to identify those fetuses that carry two copies of the defective gene long before birth so that the mother, if she wishes, can have the pregnancy terminated. In sickle-cell anemia, for example, the exact nucleotide change in the mutant gene is known (the sequence GAG is changed to GTG at a specific point in the DNA strand that codes for the β chain of hemoglobin). For prenatal diagnosis, two DNA oligonucleotides are synthesized—one corresponding to the normal gene sequence in the region of the mutation and the other corresponding to the mutant sequence. If the oligonucleotides are kept short (about 20 nucleotides), they can be hybridized with DNA at a temperature selected so that only the perfectly matched helix will be stable. Such oligonucleotides can thus be used as labeled probes to distinguish between the two forms of the gene by Southern blotting on DNA isolated from fetal cells collected by amniocentesis. A fetus carrying two copies of the mutant β-chain gene can be readily recognized because its DNA will hybridize *only* with the oligonucleotide that is complementary to the mutant DNA sequence.

For many genetic abnormalities the exact nucleotide sequence change is not known. For an increasing number of these, prenatal diagnosis is still possible by using Southern blotting to assay for specific variations in the human genome (the *RFLPs* in Figure 7–16) that are known to be closely linked to the defective gene.